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COMPARATIVE STUDY OF THE COLLEMBOLA POPULATIONS FROM A *PINUS PINASTER* AITON FOREST AND A *EUCALYPTUS GLOBULUS* LABILL. PLANTATION

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SUMMARY

In the present paper we compare Collembola populations occurring in coppices of *Pinus pinaster* Aiton, (1789) and stands of *Eucalyptus globulus* Labill., (1800) in Sever do Vouga, central Portugal. The overall data analysis (using both diversity and similarity measures) did not show clear signs of rupture on the structure of collembola populations as a consequence of eucalyptus reafforestation. More than 53% of the species are common to both sites, therefore, there is not a distinct separation between them. Nevertheless, fauna associated with the *Eucalyptus globulus* plantation is more abundant than the one connected with the natural forest which, however, is richer and more diverse than the fauna of the allochthonous stand. Data also reflect a faunal stratification between organic and mineral horizons on both sites with higher number of species and individuals occurring in the organic horizon in preference to the mineral horizon. These findings can be related to the reduced anthropogenic influence on the eucalyptus plantation since it does not follow the usual exploitation procedures, leading to a large accumulation of organic debris which probably allowed the establishment of suitable ecological conditions for collembola (specially food and microhabitat availability).

INTRODUCTION

This study was a part of a STEP-EU project entitled "Fluctuation of Biodiversity patterns following reafforestation with indigenous *versus* exotic tree species". Its aim is to compare the biodiversity and community structure of the Collembola fauna from a *Pinus pinaster* forest and a *Eucalyptus globulus* plantation. Among soil mesofauna, Collembola are usually selected as biological material for ecological studies due to their numerical representation in soil arthropod fauna (they are the second most abundant *taxon* of soil arthropods) and to the important role they play in soil processes, namely in the decomposition of organic debris. They are very sensitive to environmental changes which made them excellent indicators of the ecological status of soils (1, 2, 5, 6, 14, 18, 19). Previous studies concerning the effects of reafforestation on the edaphic fauna have indicated that these practices induce a clear rupture in the equilibrium of the collembola taxocenosis (1, 2, 5, 6, 11, 12, 13, 23). In Portugal the area planted with *Eucalyptus globulus*, an exotic tree species native from Australia and Tasmania, has been rapidly increasing during the last decades, reaching now an extension of 539 000 hectares (22). As an evergreen fast growing species it can induce changes in soil processes (water and nutrients cycles) and in the structure of soil biota connected with litter decomposition, leading to a perturbation of the equilibrium in soil system. For this reason the massive eucalyptus plantation may be considered a potential environmental problem (23). This expansion of eucalyptus distribution area, undertaken mainly by cellulose industries for economical reasons, has implicated a decrease in the area of Portuguese autochthonous forests which have been partially occupied by this exotic species. The area planted with *Pinus pinaster* (pine forests), an autochthonous species of major importance for its geographical representation and commercial value, was the most affected since this was the main species replaced by eucalyptus. The area of pine forests, concentrated in the centre littoral region of the country, has decreased to a present extension of 1050 000 hectares (22).

Given the relevance of these facts, we tried to evaluate the effects of reafforestation with *E. globulus* on the edaphic collembola populations comparing the communities from a *P. pinaster* forest and an *E. globulus* plantation.

MATERIALS AND METHODS

1. Study sites

The sampling station is located in Sever do Vouga, district of Aveiro, about 80 km north of Coimbra, centre littoral region of Portugal. The climate of the area is mesothermic and humid, meaning that water deficiency during Summer months is very low. In pedological terms, it is a region of distric cambisoils laying over schists, associated with eutric cambisoils in lower regions.

In this station we selected two contiguous stands of Pinus *pinaster* and *Eucalyptus globulus*, both situated at an average altitude of 300 m, the pine wood presenting an exposition of N 120° E and the eucalyptus plantation an exposition of N 235° E; their slopes were 15 % and 17,5 %, respectively.

Before 1973 the whole area was occupied with pine wood which was then devastated by a forest fire. Reafforestation was done in 1977 and trees (*Pinus pinaster* and *Eucalyptus globulus*) were planted in grooves opened along the altitude lines. In 1989 eucalyptus were cut down and after that the plantation remained untouched until the sampling date (March 1992), allowing the development of a vegetation cover composed of a relatively dense shrub layer dominated by *Chamaespartium tridentatum* (L.) Gibbs, *Erica* sp., *Calluna vulgaris* (L.) Hull and a herbaceous layer composed mainly of *Pteridium aquilinum* (L.) Kunh, some weed and gramineous plants. In pine wood the shrub layer was composed of *Chamaespartium tridentatum* (L.) Gibbs, *Ulex europaeus* Lineu, (1753) and *Erica* sp., the herbaceous layer included *Lithodora prostrata* (Loisel) Griseb, (1844) and gramineae. On both stands there was leaf litter accumulation on the grooves, particularly in eucalyptus plantation, where bark, branches and leaves resulting from the cut were left in place.

2. Sampling, soil characterization and fauna extraction

In each biotope one sampling quadrat (8m per 8m) was selected and then divided into 16 small squares of 2m per 2m. In the middle of each square, after a careful removal of the leaf layer, two samples of 250 cm^3 each were taken at different depths corresponding to organic and mineral horizons (designated by litter and soil, respectively) thus making a total number of 32 samples. One additional pitfall trap was placed in each square for one week in order to catch epigeic fauna.

For each sample several edaphic parameters were measured such as temperature at 10 cm soil depth, organic horizon thickness and water content according to Dewis and Freitas (8). Also evaluated were pH in distilled water (1:6 v/v) at 20 °C, organic matter content (expressed as Ash Free Dry Weight) and Carbon and Nitrogen contents, measured by Anne's and Kjeldahl's methods, respectively (8).

According to table I all parameters, except pH, have higher values on the eucalyptus stand though statistical difference is found only in pH, temperature and moisture.

This resemblance between sites is probably due to the fact that the eucalyptus plantation is not subject to the usual exploitation procedures involving regular cleaning of the soil with removal of vegetation cover and remains of plant material resulting from the cut of the trees. This led to an accumulation of organic debris and therefore an increase in soil standing stock biomass causing an increase in organic horizon thickness, nutrient levels and water content of the soil.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERIZATION OF STUDY SITES (MEAN± SD).SAMPLES WERE CUMULATED AND MEANS WERE COMPARED BY T-TEST.

	Pinus pinaster	*Eucalyptus globulus*	
Humus horiz. thickness (cm)	1,100±0,266	1,600±0,802	n.s
Soil pH	4,200±0,234	3,800±0,175	**
Soil temperature (°C)	10,800±0,856	14,200±1,940	*
% Water content (humus)	20,320±12,788	36,040±18,157	***
% Water content (soil)	25,300±6,560	27,790±10,342	n.s
% Carbon (soil)	6,719±3,892	9,231±5,334	n.s
% Nitrogen (soil)	0,273±0,161	0,352±0,205	n.s
C/N (soil)	24,869±1,758	31,938±28,499	n.s
% Org matter (humus)	38,900±20,586	50,312±24,378	n.s
% Org. matter (soil)	11,900±7,061	13,481±7,052	n.s

$^{*}p<0,0001$ $^{**}p<0,01$ $^{***}p<0,05$ n.s - non significant

Soil microarthropods were extracted using the Berlese-Tullgren funnels method and the Collembola sorted, under binocular microscope, and identified, under light microscope, at species level.

3. Data analysis

For both sites the total number of samples at each stratum (organic and mineral horizons and pitfall traps) were grouped, making 6 groups for 47 *taxa*. This way a global data matrix was obtained showing the amounts of the several species found in each stratum. Two different methodologies were then used to compare the composition of both collembolan communities: diversity and similarity measures.

To evaluate the diversity of both communities we calculated, for each stratum, species diversity, evenness and species richness using the indices of Shannon-Weaver, Pielou and

Margalef, respectively (15). As a second analytical approach we undertook a numerical analysis to search for patterns of affinity between samples and between the species. For this purpose data from the original matrix were transformed to Log (xi+1) and the Bray Curtis coefficient, selected for its robustness in analysing ecological data (10), was applied to the resulting matrix in order to measure the structural similarity of the communities from the two sites. Two similarity matrixes were then obtained allowing the performance of an agglomerative hierarchical clustering analysis which produced the phenograms of Q mode, or normal analysis and R mode, or inverse analysis, displaying graphically the relations between strata and between species, respectively. Finally, a "nodal analysis", a post-clustering regular procedure aimed to enhance the interpretation of the numerical classification results, was done. This procedure, which is based on the ecological concepts of Constancy and Fidelity, relates the groups of similar samples defined by normal analysis to groups of co-occurrence *taxa* defined by inverse analysis (4). Constancy and Fidelity are given by indices, following Boesch (4) and numerically expressed as:

- $C_{ij} = a_{ij} / (n_i n_j)$ for Constancy
- $F_{ij} = (a_{ij} \Sigma n_j) / (n_j \Sigma a_{ij})$ for Fidelity

where a_{ij} is the actual number of occurrences of members of species group i in the samples group j and the n_i and n_j are the numbers of entities in the respective groups. Constancy index has a maximum of 1 when all species occurred in all samples in the group and a minimum of 0 when none of the species occurred in that particular samples group. Fidelity index expresses the constancy of species in a group of samples in relation to the constancy of the same species in all groups of samples. Fidelity values greater than 2 suggest strong "preference" of species for a samples group and values lower than 1 suggest that species "avoid" the habitats corresponding to the samples group, or negative fidelity (4).

RESULTS

A total number of 47 *taxa* were identified among 4040 individuals found in both biotopes (table II). Eucalyptus plantation registered the highest abundance of individuals being represented by 2940 specimens distributed by 35 *taxa*, while pine wood had the higher number of species, being represented by 37 *taxa* and 1100 specimens. In both biotopes the litter layer presents higher number of species and individuals than the soil layer.

Differences between autochthonous and allochthonous sites were also found considering diversity measures (table III). The comparison of the corresponding strata from both biotopes shows that pine wood presents higher species diversity values in litter and pitfall samples than eucalyptus stand which, however, has the highest value in soil samples. This superiority in

TABLE II

TOTAL ABUNDANCE OF COLLEMBOLAN SPECIES IN COPPICES OF PINE WOOD AND EUCALYPTUS PLANTATIONS

	Pinus pinaster			*Eucalyptus globulus*		
Taxa	Litter	Soil	Pitfall	Litter	Soil	Pitfall
Xenylla franzi Steiner, 1955	151	7	1	795	53	4
Xenylla schillei Borner, 1903	5		1	1		
Pseudachorutella asigillata (Borner, 1901)				1		
Pseudachorutes subcrassus Tullberg, 1871		1		23	1	3
Rusekella gamae Arbea et Jordana, 1991				2		
Micranurida sp. (2+2 eyes)	65	2		94	13	
Superodontella carmenae Arbea et Luciáñez, 1991		3		1		
Bilobella aurantiaca (Caroli, 1910)	11	3		42	5	
Deutonura portucalensis (Gama, 1964)	2	1		2	4	
Onychiurus armatus (Tullberg,1869), Gisin, 1952				9	14	
Onychiurus circulans Gisin, 1952	3	8			1	
Mesaphorura arbeai Simón, Ruiz, Martin et Luciáñez	5	2		10	34	
Mesaphorura coimbrensis n. sp.	12			16	69	
Mesaphorura macrochaeta Rusek, 1976	8	6				
Mesaphorura isochaeta Arbea et Jordana,1989					2	
Mesaphorura yosii Rusek, 1976	2				33	
Mesaphorura sp.	2	6		3	10	
Paratullbergia callipygos (Börner), 1902	1	2				
Proctostephanus madeirensis Gama, 1959				6	48	
Tetracanthella hygropetrica Cassagnau, 1954	122	93		875	306	
Tetracanthella proxima Steiner, 1955	1			5	11	
Proisotoma coeca Gama, 1961		1				
Cryptopygus scapelliferus (Gisin, 1955)	3					
Isotomurus fucicola (Reuter, 1891)	5	7				
Isotomiella minor (Schaffer, 1896)					1	
Isotoma notabilis (Schaffer, 1896)					1	
Sinella sp. (4+4 eyes)	1	1		1	2	
Entomobrya albocincta (Templeton, 1835)	7		1			
Entomobrya multifasciata (Tullberg, 1871)	6			37	3	21
Entomobrya cf. muscorum (Nicolet, 1841)	17	1	4	9	1	3
Heteromurus major (Moniez, 1889)	62	2	9	51	7	4
Lepidocyrtus paradoxus Uzel, 1890	12	1	9		1	4
Lepidocyrtus sp.	81	18	18	41	4	6
Pseudosinella picta (Borner, 1903)	6	2		25	16	
Pseudosinella sp.				7		
Pogonognathellus flavescens (Tullberg, 1871)	31		2	5		
Tomocerus vulgaris (Tullberg, 1871)	4		1	4		1
Entomobryidae juv.	235	6	1	108	19	5
Megalothorax minimus Willem, 1900	4	1				
Sminthurides parvulus (Krausbauer, 1898)			1			
Sphaeridia pumilis (Krausbauer, 1898)	1		1			
Arrhopalites sericus Gisin, 1947				6		
Caprainea bremondi Delamare et Bassot, 1957	3			2		
Dicyrtomina minuta (O. Fabricius, 1783)	1		2			
Bourletiella gamae Bretfeld, 1994				4	3	42
Sminthuridae juv.	2					

litter samples from pine wood in relation to eucalyptus is mainly due to differences in the corresponding evenness values since the differences in the number of *taxa* between sites are small. Thus, a lower species diversity on eucalyptus litter reflects lower evenness values which are related to the high abundance of two species: *Tetracanthella hygropetrica* and *Xenylla franzi*. In soil samples the difference is explained not by the eveness values, which are very similar, but by the higher number of *taxa* in eucalyptus.

Pine wood shows the highest species richness values in all strata. This is due to the lower number of specimens in this biotope since the differences in species number between sites is very small.

TABLE III

DIVERSITY, EVENESS AND SPECIES RICHNESS FOR THE DIFFERENT STRATA OF BOTH BIOTOPES

Biotopes	*Pinus pinaster*			*Eucalyptus globulus*		
Strata	litter	soil	"pitfall"	litter	soil	"pitfall"
Diversity	3.385	2.866	2.847	2.416	2.970	2.460
Eveness	0.677	0.634	0.769	0.497	0.632	0.741
Richness	4.579	4.246	3.052	3.641	3.849	1.986
# "Taxa"	32	23	13	29	26	10
# Individuals	871	178	51	2185	662	93

= Number

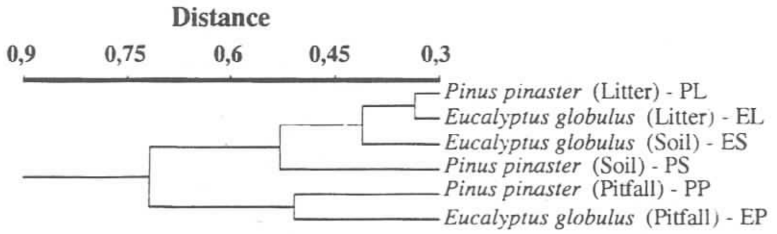
Distance
0,9
0,75
0,6
0,45
0,3
Pinus pinaster (Litter) - PL
Eucalyptus globulus (Litter) - EL
Eucalyptus globulus (Soil) - ES
Pinus pinaster (Soil) - PS
Pinus pinaster (Pitfall) - PP
Eucalyptus globulus (Pitfall) - EP

FIGURE 1. Phenogram corresponding to the Q mode analysis based on Bray-Curtis distances.

In the phenogram of R analysis (fig. 2) 10 different groups (A to J) can be distinguished based on the presence and/or species abundance on samples. More than 53% of the species

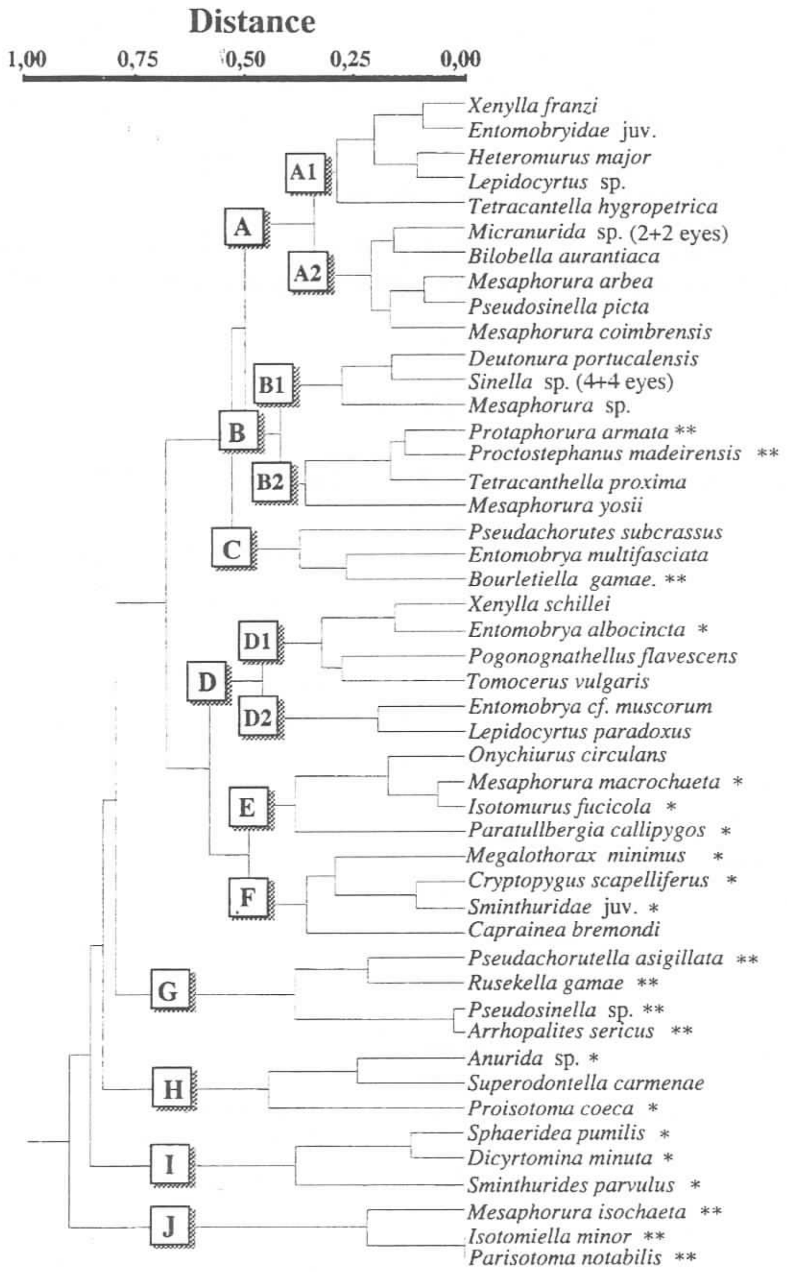

FIGURE 2 - Phenogram corresponding to the R mode analysis based on Bray-Curtis distances. "*" signs species occurring only in *P. pinaster* site and "**" signs exclusive species of *E. globulus* site.

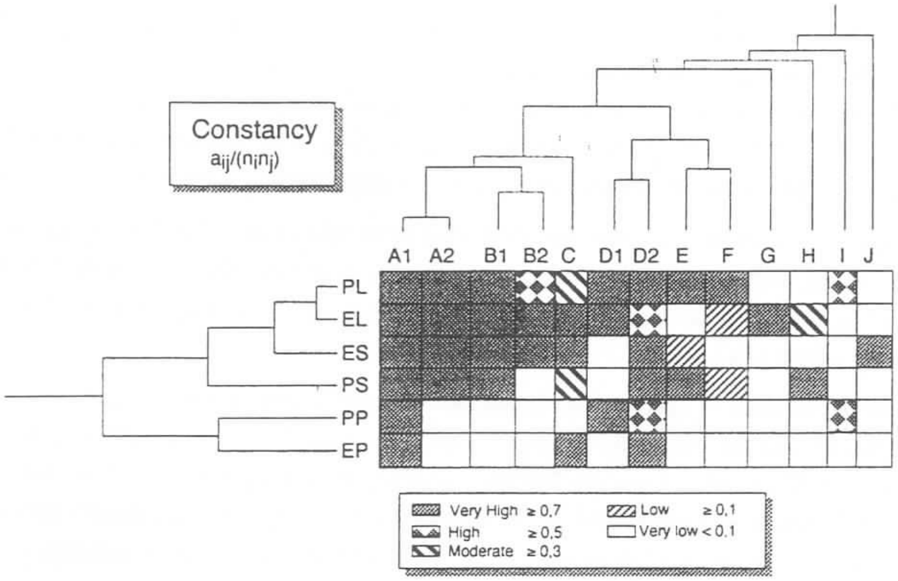

FIGURE 3. Constancy values for post-clustering nodal analysis. Samples and species groups according to figures 1 and 2, respectively.

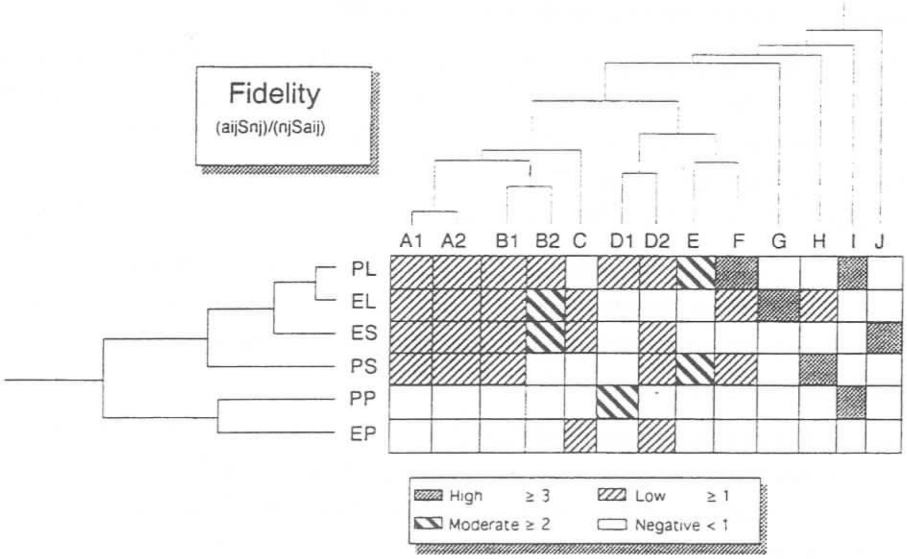

FIGURE 4. Fidelity values for post-clustering nodal analysis. Samples and species groups according to figures 1 and 2, respectively.

In the phenogram of R analysis (fig. 2) 10 different groups (A to J) can be distinguished based on the presence and/or species abundance on samples. More than 53% of the species found are present in both biotopes including the most abundant, thus, the majority of the groups (A, B, C, D, E, F and H) have species which are common to both sites. The groups G and J show exclusivity for eucalyptus and the group I is exclusive from pine wood.

Cluster A is composed by 10 *taxa* and it's divided in the subclusters A1 and A2. Subcluster A1 presents very high constancy indices in all strata (fig. 3) and therefore low fidelity values for them (fig. 4). A2 includes species absent from pitfall samples with patterns of constancy and fidelity similar to A1.

Cluster B includes 7 species not represented in pitfalls and is also divided in two subclusters, B1 and B2. Subcluster B1 follows patterns of constancy and fidelity similar to subcluster A2; B2 includes 4 species, 2 of them showing exclusive preference for eucalyptus (*Protaphorura armata* and *Proctostephanus madeirensis*) either in litter and soil samples (report also table I), the other 2 although present in pine wood litter are more constant in eucalyptus, showing some preference for the corresponding strata.

Cluster C includes 3 species, one of them exclusive from eucalyptus (*Bourletiella gamae*), the other 2 are common to both biotopes (only absent from pine wood pitfalls) preferring eucalyptus stand samples where their constancy is also higher.

Cluster D is formed by 6 species, one of them exclusive from pine wood (*Entomobrya albocincta*), and is divided in subclusters D1 and D2. Subcluster D1 includes 4 species showing higher constancy values on litter samples of both biotopes and on pine wood pitfalls, presenting here a moderate fidelity. The 2 species of subcluster D2 present high constancy values together with low fidelity values in all strata.

Cluster E has 4 species, 3 of them found exclusively in pine wood litter and soil samples (*Mesaphorura macrochaeta*, *Isotomurus fucicola* and *Paratullbergia callipygos*) where they are very constant; the fourth species is found in eucalyptus soil though poorly represented there (a single individual - see table I).

Clusters F to J include species scarcely represented. Cluster F is composed by 4 species, 3 of them exclusive from pine wood (*Megalothorax minimus*, *Cryptopygus scapelliferus* and *Sminthuridae* juv.), the other species is found only in eucalyptus litter (table I). They all occur preferentially in pine wood litter, according to the corresponding fidelity values, where its constancy is very high. Cluster G has 4 species showing exclusive preference for eucalyptus litter (*Pseudachorutella asigillata*, *Rusekella gamae*, *Pseudosinella* sp and *Arrhopalites sericus*) therefore, the corresponding values of constancy and fidelity are high. The 3 species of H cluster show high fidelity values tpine wood soil, 2 of them being exclusive from this stratum (*Anurida* sp. and *Proisotoma coeca*). The third species is also represented by a single individual in eucalyptus litter (table I). Group I includes 3 species which are exclusive from pine wood litter and pitfall samples and, finally, group J includes 3 species presenting absolute fidelity to eucalyptus litter.

DISCUSSION

The global analysis of the obtained results does not show clearly that reafforestation with eucalyptus has led to a rupture in the collembola biocenosis balance. Contrasting to the results of previous studies regarding the impact of eucalyptus cultures on the edaphic collembola (11, 12, 23) there is not, in this case, a distinct separation between the fauna associated to the autochthonous and the allochthonous tree species although important differences were observed.

Eucalyptus plantation has much higher number of individuals than pine wood, which is probably and mainly explained by the higher water content of the eucalyptus soil specially in the litter layer due to water retention by the large amounts of accumulated organic matter. This is the most probable explanation, since moisture is one of the most important ecological factors controlling the distribution of these animals in the edaphic system, being higher number of individuals associated to microhabitats with higher water content (21).

Though the higher number of specimens and, therefore, the higher number of preferential species is found in eucalyptus, the pine wood community presents, in a general way, higher species richness, evenness and diversity values together with a higher number of exclusive species. These findings are identical to the results obtained by the authors mentioned above (23), who have found for these parameters higher values in the natural forest, although, in this case, differences in total number of species and exclusive species are small.

In both sites abundance data show a vertical stratification of the communities since the number of both individuals and species is higher in litter in detriment of soil layer. This is a very common event in undisturbed soils of temperate regions, also reported by authors like Gama *et al* (12), Mateos (17), Pozo and Martinez (20) and Sousa and Gama (23).

It is known that the major part of organic debris colonised by the microflora, which collembola feed on, is concentrated in upper soil layers and that with the increase of soil depth there is a reduction of soil porosity (7, 9, 16) providing pore spaces critical living space or appropriate moisture levels for soil arthropods (3). Thus, this stratification is probably due to the fact that litter layer provides the best ecological conditions for the individuals needs, namely in terms of food and microhabitat availability, possibly also reflecting a microclimatic stratification between horizons (layers).

The absence of a clear separation between biotopes is pointed out by the phenogram of Q analysis which shows a higher degree of association between the corresponding strata of both sites, namely concerning pitfall and litter samples, being pine wood litter closer to eucalyptus litter and soil than to the corresponding soil.

R analysis confirms the affinity between both communities in terms of species composition. Subclusters A1 (which includes 76,3% of total number of individuals), A2, B1 and D2 are very constant in all strata of both biotopes (except subclusters A2 and B1 for pitfall), subclusters B2 and D1 also reveal high constancy values in both biotopes though being more constant in eucalyptus and pine wood, respectively. In the other groups, however, lower constancy values are observed, with significant differences between strata. Thus, cluster C is more connected with eucalyptus since it shows very high constancy values in all strata of this biotope, which is not observed in pine wood where the group presents only a moderate constancy. Groups E, F and H are mainly attached to pine wood as they exhibit in some of these strata higher values of constancy than in eucalyptus. Most of the species present low fidelity for all strata from both sites, except those which belong to the exclusive groups (G, I and J) and to subgroups B2 and D1 and groups E, F and H, dominated by exclusive species which actually present higher fidelity to some of the strata.

So, this analysis does not reveal a clear separation of the collembolan communities from pine wood and eucalyptus plantation since most of the species, including the most abundant, are common to both sites. On the other hand, even the species groups attached to each biotope are mainly due to the presence of exclusive species which are rare and represent a small portion of total species found in both communities.

Reafforestation, involving replacement of autochthonous plants by eucalyptus, usually causes the establishment of new ecological (change in abundance and composition of microflora, in the availability of trophic niches and microhabitats, etc.) physical and chemical conditions in soils. These changes, which result not only from the new introduced species but also from the technical procedures used to plant the trees and manage the plantation, affect soil communities leading to a rupture in their balance (decrease in total number of individuals and species and decrease in diversity indices). However, as previously mentioned, the eucalyptus plantation from Sever do Vouga is not subject to the usual exploitation procedures. This, together with the fact that trees were planted in grooves, led to an accumulation of organic debris, allowing apparently the establishment of favourable ecoclimatic conditions to the maintenance and growth of the collembolan populations, namely considering the availability of trophic niches and microhabitats.

The absence of a clear distinction between both communities reflects, therefore, the existing homogeneity between the two biotopes as a consequence of a very unusual case of management in the eucalyptus stand. These results make us realize how important the plantation exploitation system can be on the evaluation of eucalyptus influence over soil living organisms regarding, for instance, that a previous work done by Sousa and Gama (23) has pointed out that disruptions in the community composition from eucalyptus would partially be explained by a soil surface management (resulting from the exploitation procedures in the plantation) that led to soil impoverishment with decrease of food and moisture levels.

Anyway, the effects on soil fauna are only a small part of the question and thus further investigations over the subject are justified and necessary, searching for data obtained in different conditions, in order to reach a higher level of knowledge about the nature and dimension of the environmental effects of eucalyptus plantations which due to their increasing expansion in these past years have been subject of great controversies between economists and ecologists in our country.

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